fluences only the extent of binding of enzyme with substrate, if K_{M} is accepted as a measure of the binding.[23] The strength of the binding of Ca^{II} to the enzyme suggests chelation, which in turn suggests that Ca^{II} exerts its effect on the kinetics by stabilizing configurations favorable for binding of the substrate.[24,25]

(23) The evidence is summarized by K. J. Laidler, *Disc. Faraday Soc.*, **20**, 83 (1955).

(24) I. M. Klotz in "The Mechanism of Enzyme Action," W. D. McElroy and B. Glass, eds., Johns Hopkins Univ. Press, Baltimore, Md., 1954.

(25) It is possible that addition of Ca^{II} reduces the extent of enzyme–enzyme complexing, thereby raising the rate. However, this would increase k_3 rather than reduce K_{M} and could not amount to more than a few per cent. at the enzyme concentration employed. Judging by the flatter *p*H *vs.* time curves, Ca^{II} does seem to protect γ-chymotrypsin from inactivation. A similar stabilization by calcium chloride has been reported for α- and B-chymotrypsin.[22]

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[CONTRIBUTION NO. 1022 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

Chelating Properties of β-Mercaptopropionic Acid

BY QUINTUS FERNANDO AND HENRY FREISER

RECEIVED APRIL 4, 1958

The acid dissociation constants of β-mercaptopropionic acid and the stability constants of its chelates with zinc(II) and nickel(II) have been determined potentiometrically by the Calvin–Bjerrum method. The position of zinc(II) in the usual stability sequence of divalent metals has been found to be altered. The chelates of β-mercaptopropionic acid are much less stable than the corresponding chelates of mercaptoacetic acid. This decrease in stability in going from five- to six-membered chelate rings is a general phenomenon which with sulfur present in the chelate ring seems much more pronounced. The reactions of β-mercaptopropionic acid with metal ions have been investigated and the chelates of silver(I), copper(II) and lead(II) have been prepared. The polarographic behavior of β-mercaptopropionic acid has been examined and compared with that of mercaptoacetic acid.

The stability sequence for metal chelates is essentially the same for a large number of chelating agents.[1] In the relatively few cases where the stability of sulfur-containing compounds has been determined, significant alteration of this sequence has been observed. For example, in a comparative study of the chelates of *o*-aminophenol and *o*-aminobenzenethiol,[2] the latter compound was found to give more stable chelates, and the stability order of the divalent metal chelates showed some differences from the usual stability sequence. Also, in the case of mercaptoacetic acid complexes the zinc complex had an anomalously greater stability than the nickel complex.[3] This work on β-mercaptopropionic acid was carried out in order to investigate further the effect of the sulfur atom on the stability of metal chelates.

Experimental

Materials.—β-Mercaptopropionic acid, kindly supplied by Evans Chemetics, N. Y., was found to be 99.85% pure by potentiometric titration with standard sodium hydroxide at 25° in an atmosphere of nitrogen.

In all *p*H measurements, a glass-saturated calomel electrode pair and a model G Beckman *p*H meter, standardized with a potassium acid phthalate buffer at *p*H 4.01 was used.

The compound was found to be quite stable, and aqueous solutions when stored in well stoppered flasks showed no appreciable deterioration for several weeks.

One per cent. aqueous solutions of the common metal ions were prepared from reagent grade salts and used for qualitative tests.

The preparation and standardization of metal perchlorate solutions and of standard sodium hydroxide used in the determination of chelate formation constants have been previously described.[4]

Buffer solutions of ionic strength 0.2 were made with boric acid and sodium hydroxide, disodium hydrogen phosphate and sodium dihydrogen phosphate, acetic acid and sodium acetate, disodium hydrogen phosphate and citric acid, together with appropriate quantities of potassium chloride. All these buffer components were examined polarographically for reducible impurities before use in the polarographic determinations.

Reactions with Metal Ions.—When a 1% aqueous solution of β-mercaptopropionic acid was added to solutions containing various cations, the following observations were made. Nickel(II) formed a deep red color.[5,6] Cobalt(II) gave a deep green color in the presence of ammonia[7]; this solution gradually turned brown on standing due to air oxidation. Iron(III) gave a transient blue color and a white precipitate which was found to be dithiodipropionic acid. Iron(II) gave a blue color which faded very rapidly. Silver(I) and mercury(II) gave white precipitates of the metal complexes soluble in ammonia. Lead(II), copper(II) and bismuth(III) formed pale yellow precipitates which also dissolved in ammonia. Cadmium(II) formed an unstable white precipitate which was soluble in ammonia as well as in excess β-mercaptopropionic acid.

Preparation of β,β′-Dithiodipropionic Acid $\text{HOOC(CH}_2)_2\text{SS(CH}_2)_2\text{COOH}$.—An attempt was made to prepare this compound by the air oxidation of β-mercaptopropionic acid. After passing air through an aqueous solution of the compound for about eight hours no precipitate was obtained. This shows that β-mercaptopropionic acid is very much more stable than thioglycolic acid, which is readily oxidized in air to dithiodiglycolic acid.[8]

On adding a solution of ferric chloride in dilute sulfuric acid to an aqueous solution of β-mercaptopropionic acid, a greenish-blue color was formed and after several minutes a white precipitate was obtained. The addition of the ferric chloride was continued with stirring until an excess of ferric ions was present. The solution was allowed to stand with stirring for about an hour and the precipitate filtered, washed with water, and recrystallized from ethanol; m.p. 151°.

Anal. Calcd. for $C_6H_{10}O_4S_2$: C, 34.27; H, 4.79. Found: C, 34.22; H, 4.55.

Preparation of Chelates.—To aqueous solutions of copper(II), lead(II) and silver(I) nitrates, perchlorates or acetates, an excess of β-mercaptopropionic acid was added with stirring. Precipitates were formed and were allowed to stand

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(1) D. P. Mellor and L. E. Maley, *Nature*, **161**, 436 (1948).

(2) R. G. Charles and H. Freiser, THIS JOURNAL, **74**, 1385 (1952).

(3) D. L. Leussing, Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March, 1958.

(4) H. Freiser, R. G. Charles and W. D. Johnston, THIS JOURNAL, **74**, 1383 (1952).

(5) L. J. Uhlig and H. Freiser, *Anal. Chem.*, **23**, 1014 (1951).

(6) J. B. Lear and M. G. Mellon, *ibid.*, **25**, 1411 (1953).

(7) E. Lyons, *ibid.*, **27**, 1813 (1955).

(8) D. L. Leussing and I. M. Kolthoff, *J. Electrochem. Soc.*, **100**, 334 (1953).

for about an hour and then filtered and washed several times with distilled water, dried in a vacuum desiccator and finally in an oven at 110°.

When the yellow copper complex was dried in air it gradually turned green. A sample of the yellow compound was isolated for analysis by filtering in an atmosphere of nitrogen and rapidly drying it in an oven at 110°. The yellow and green complexes of copper were interconvertible. When β-mercaptopropionic acid was added to the green complex, it turned yellow. If pyridine was added to the green compound, a green solution of the copper–pyridine complex was obtained and the yellow copper complex precipitated. The green copper complex was also soluble completely in 1:1 ammonia. The metal complexes were analyzed.

Anal. Calcd. for $Pb(SCH_2CH_2CCO)$: Pb, 66.56. Found: Pb, 66.16. Calcd. for $Ag(SCH_2CH_2COO)^- \cdot H_3O^+$: Ag, 46.69. Found: Ag, 46.60. Calcd. for $Cu(SCH_2CH_2COO)$: Cu, 37.91. Found: Cu, 38.03 pale green. Calcd. for $Cu(HSCH_2CH_2COO)_2$: Cu, 23.22. Found: Cu, 21.90 yellow.

Chelate Formation Constants.—The titration apparatus has been described in detail previously.[4] The procedure adopted was essentially the same as that described earlier. One hundred ml. of water and 5 ml. of metal perchlorate (0.01 *M*) solution were added to a weighed amount of reagent in the titration vessel and titrated with standard sodium hydroxide (0.1 *N*), with an atmosphere of nitrogen maintained above the solution during the titration. The titration was carried out with the reagent alone, in a similar manner, after the addition of 5 ml. of water instead of the metal perchlorate solution. All *p*H measurements were made with a Beckman model G *p*H meter equipped with a glass and saturated calomel electrode. Figure 1 shows the titration curves obtained. The ratio of reagent and metal ion concentration was varied from 6:1 to 12:1, and the reported values of the chelate formation constants are an average of five determinations for each metal ion.

If K_1 and K_2 are the acid dissociation constants of the carboxylic group and the mercapto group, respectively, in β-mercaptopropionic acid, it can be shown that $\bar{n}$, the average number of anions $(SCH_2CH_2COO)^{--}$ bound to a divalent metal ion is given by

$$\bar{n} = \frac{1}{T_m}\left[T - S\left(\frac{K_1 + H^+}{K_2 + H^+}\right)\right]$$

where T_m = total metal ion concentration, T = total reagent concentration and $S = 2T - Na^+ + OH^- - H^+$. The stepwise formation constants for the chelates of zinc(II) and nickel(II) were obtained by plotting $\bar{n}$ against $-\log R$, where

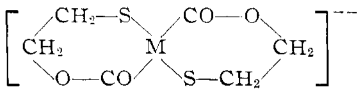
$$R = [(SCH_2CH_2COO)^{--}] = \frac{SK_1K_2}{H^+(K_1 + 2H^+)}$$

The values of $-\log R$ at $\bar{n} = 0.5$ and $\bar{n} = 1.5$ gave the stepwise formation constants.

Polarography.—A Leeds and Northrup model E electrochemograph was used to record all polarograms. Measurements were made in an H-type cell with a saturated calomel electrode as reference electrode. The cell was maintained at 25 ± 0.05°. The characteristics of the capillary used were m = 3.128 mg./sec. and t = 3.05 sec. measured in a disodium hydrogen phosphate–citric acid buffer at *p*H 6.30 and an applied potential of −0.35 volt *vs.* S.C.E. with the mercury column 50 cm. high. Oxygen was removed from all solutions with nitrogen. A voltage span of 2 volts was used in all experiments with the damping switch at zero. No corrections were found to be necessary for the *iR* drop since the cell resistance was small. In all cases the average current values were measured.

Results and Discussion

Chelates.—β-Mercaptopropionic acid reacts with metal ions to form precipitates or anionic complexes. Two moles of the reagent combine with one mole each of zinc(II), nickel(II) and cobalt(II) to form complexes of the type



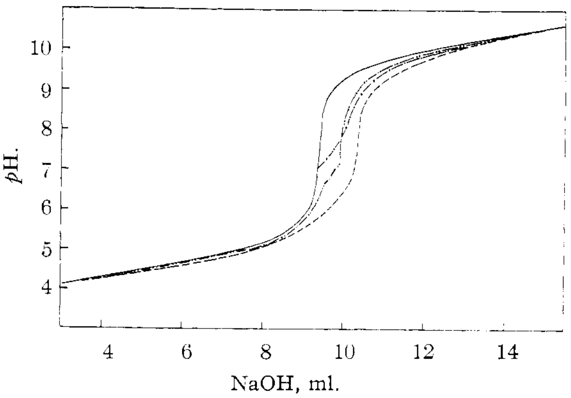
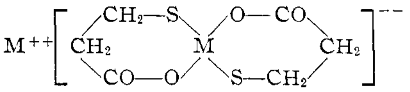


Fig. 1.—Titration curves for β-mercaptopropionic acid at 25°: ———, no metal; –·–·–·, Ni(II); ··–··–, Co(II); ---------, Zn(II).

The zinc complex is colorless, the nickel complex red and the cobalt complex green. With lead (II) and copper (II) stable precipitates containing one mole of reagent per mole of metal ion were obtained. These compounds can be formulated as



The silver (I) complex is possibly $Ag(SCH_2CH_2COO)^- \cdot H_3O^+$, but the formula of the yellow copper (II) complex is uncertain since it could not be isolated in a sufficiently pure condition to give a reliable analysis. This behavior of copper(II) with β-mercaptopropionic acid is unusual since with thioglycolic acid, copper(II) is reduced to copper(I) with the formation of dithiodiglycolic acid.[3]

Stability Constants.—Figure 1 shows the potentiometric titration curves for zinc(II), nickel(II) and cobalt(II). Table I gives the acid dissociation constants and the stepwise formation constants for the zinc and nickel complexes. No attempt was made to calculate the formation constants for the cobalt complex, since oxidation of the cobalt(II) to cobalt(III) occurred during the titration in spite of the nitrogen atmosphere maintained in the titration vessel.

TABLE I

ACID DISSOCIATION CONSTANTS AT 25° IN WATER

Acid	pK_1	pK_2
Mercaptoacetic[3]	3.60	10.55
β-Mercaptopropionic	4.38	10.38

CHELATE FORMATION CONSTANTS AT 25° IN WATER

	Mercaptoacetic acid[3]			β-Mercaptopropionic acid		
	$\log k_1$	$\log k_2$	$2 \log k_{av}$	$\log k_1$	$\log k_2$	$2 \log k_{av}$
Nickel(II)	6.98	6.55	13.53	5.21	4.39	9.60
Zinc(II)	7.86	7.18	15.04	6.75	6.05	12.80

Zinc forms more stable complexes than nickel. This alteration in the usual stability sequence of the divalent metals also has been observed in the case of mercaptoacetic acid.[3] Even more noteworthy is the difference between the stability of the five-membered chelate rings containing a sulfur–metal bond. In similar chelate rings in which more electronegative atoms are involved, such as oxygen or nitrogen, the differences between 5- and 6-membered rings are not as marked.

In comparing the stabilities of 5-and 6-membered chelate rings, a complicating factor is the change of the pK_a values of the ligand molecules involved in the formation of these rings. Both the acidity and basicity, in the Lewis sense, of the ligand affect the stability of the chelate formed. In order to observe the effect of ring size alone, values for the quotient $\Delta\log K_1/\Delta pK_{av}$ for corresponding 5-and 6-membered rings are compared (Table II); $\Delta\log K_1$ is the decrease in the value of the first formation constant of the chelate in going from five- to six-membered rings and ΔpK_{av} is the increase in the arithmetic mean of the negative logarithms of the acid dissociation constants of the reagent molecules involved in forming 5- and 6-membered rings. The data shown in Table II indicate that the decrease in stability in going from 5- to 6-membered rings is of the same order of magnitude for dicarboxylic acids, amino acids and diamines. However in the case of mercaptoacids, this decrease is very much greater. Since the decrease is of the same order of magnitude as that observed in going from 6- to 7-membered rings in dicarboxylic acids, this might suggest that with the bulky sulfur atom a 6-membered ring leaves about as little room for the metal ion as there is in a 7-membered ring containing oxygen. If this is the case, reagents containing two sulfur atoms should exhibit a still greater decrease in chelate stability in going from the 5-membered to 6-membered rings. The larger size of the sulfur atoms would, by the same reasoning, help explain the existence of stable 4-membered chelates such as those of the xanthates and thiocarbamates.

TABLE II

RELATIVE STABILITIES OF 5-, 6- AND 7-MEMBERED CHELATE RINGS[9]

	$\Delta\log K_1/\Delta pK_{av}$		
Metal ion	Dicarboxylic acids Oxalic and malonic	Amino acids Glycine and β-alanine	Diamines Ethylenediamine and 1,3-diaminopropane
Copper(II)	1.04	1.18	0.92
Nickel(II)	1.09	1.19	1.16
Zinc(II)	0.95	1.38	..
Cobalt(II)	0.93	..	..
	Malonic and succinic	Mercapto acids Thioglycolic and β-mercaptopropionic	
Copper(II)	2.32	..	
Nickel(II)	..	2.90	
Zinc(II)	1.94	1.82	

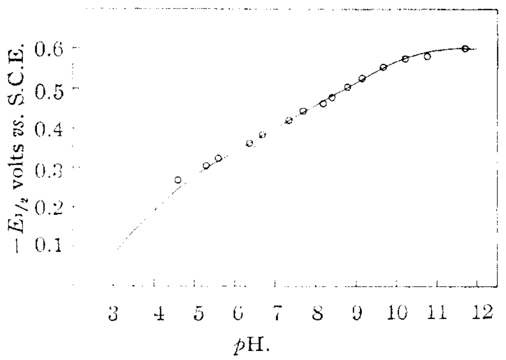


Fig. 2.—Effect of *p*H on the half-wave potential of β-mercaptopropionic acid: ———, calculated values; O, observed values.

Polarography.—Polarograms were obtained for β-mercaptopropionic acid in various buffers of ionic strength 0.20 between *p*H 11.7 and 3.0. In all solutions a well defined anodic wave was observed. At *p*H values less than 4.6, a prewave appeared, and the measurement of the wave height and the half-wave potential of the well defined anodic wave was made difficult. The variation of the half-wave potential with *p*H is given in Fig. 2. The height of the anodic wave was independent of *p*H, at *p*H values greater than 4.6, and was proportional to the concentration of β-mercaptopropionic acid up to a concentration of about 2 millimoles per liter. The diffusion current constant calculated for the wave at *p*H 6.3 in a McIlvaine buffer was 1.86. A plot of $\log(i_d - i)/i$ *vs.* the applied potential for the same wave gave a straight line of slope 0.056. These results show that the anodic oxidation taking place is a reversible single electron process.

In many respects the polarographic behavior of β-mercaptopropionic acid is similar to that of mercaptoacetic acid. Leussing and Kolthoff[8] have shown that a mercury compound is formed during the anodic oxidation of thioglycolic acid. It would

$$HSCH_2COO^- + Hg \rightleftarrows HgSCH_2COO^- + H^+ + e^-$$

be reasonable to infer that β-mercaptopropionic acid too undergoes oxidation in a similar manner. In order to test this, polarograms of β-mercaptopropionic acid were obtained with a stationary platinum microelectrode. No anodic or cathodic waves were found, thus confirming the fact that the mercury takes part in the electrode reaction as

$$HSCH_2CH_2COO^- - Hg \rightleftarrows HgS[CH_2]_2COO^- + H^+ + e^-$$

It can be shown readily that the half-wave potential of the anodic wave is given by the equation

$$E_{1/2} = E^0 + 0.059 \log\left\{\frac{[H^+]^2}{K_1} + [H^+] + K_2\right\}$$

neglecting activity coefficients, where K_1 and K_2 are the acid dissociation constants of the carboxylic and mercapto groups. The value of E^0 calculated from the experimental values of *p*H, K_1 and K_2 was +0.015 volt. Below *p*H 4.6, $E^1/_2$ values were not measured due to the interfering prewaves. Figure 2 shows the agreement between calculated and observed half-wave potentials at *p*H values greater than 4.6.

An attempt was made to determine the stoichiometry of the zinc and nickel complexes of β-mercaptopropionic acid by the addition of amounts of zinc or nickel to a solution containing the acid until the wave height of the anodic wave diminished to zero. However, the only effect that the addition of zinc had on the anodic wave was to shift the half-wave potential to less negative values. When calculated quantities of nickel were added, an anodic and cathodic wave appeared. The half-wave potential of the anodic wave was shifted to less negative values while the half-wave potential

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[9] J. Bjerrum, *et al.*, "Stability Constants, Part 1; Organic Ligands," The Chemical Society Special Publication No. 6, London (1957).

of the cathodic wave remained constant, with increasing amounts of nickel. The half-wave potential of the anodic wave of β-mercaptopropionic acid decreased by 17 millivolts for each micromole of Ni(II) or Zn(II) added. Similar results were obtained when zinc and nickel were added to solutions of thioglycolic acid. The half-wave of the anodic wave of thioglycolic acid decreased by 20 millivolts for each micromole of Ni(II) or Zn(II) added.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PENNSYLVANIA]

An Estimate of the Conformational Equilibrium in Cyclohexanol from Infrared Spectra[1]

BY ROGER A. PICKERING AND CHARLES C. PRICE

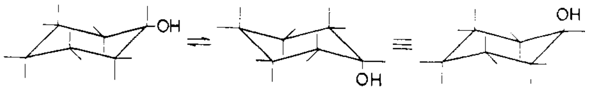
RECEIVED APRIL 2, 1958

The C–OH stretching frequency for *cis*- and *trans*-4-*t*-butylcyclohexanol is empirically assigned to the strong absorption occurring at 955 and at 1062 cm.$^{-1}$, respectively. By comparing the intensity of absorption of cyclohexanol at 1069 cm.$^{-1}$ with that of the *trans* isomer at 1062 cm.$^{-1}$, it may be estimated that cyclohexanol exists 62.6–66.2% in the equatorial conformation. This estimate, based on the assumption of equal absorbance for the equatorial hydroxyl group in cyclohexanol and its *trans*-4-*t*-butyl homolog, gives a value for the free energy difference between the equatorial and axial conformations for the hydroxyl group in cyclohexane (0.3–0.4 kcal./mole) which is lower than other estimates.

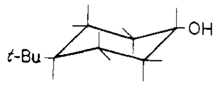
Introduction

In recent years the concept of conformational analysis has been established as an approach of great value in the solution of a wide variety of problems in structural organic chemistry.[2] Specifically, it has been shown that a substituent on a cyclohexyl ring can be bonded in two ways—axially or equatorially—in the favored chair conformation and that the reactivity of the substituent will be affected accordingly. The principles of conformational analysis having been established chiefly through studies of fused ring systems of rigid conformation such as terpenes and steroids, it has been a problem of some interest to extend the approach to those simpler cyclohexane derivatives which are conformationally mobile.

Winstein and Holness[3] and Eliel and co-workers[4] have approached the problem from the standpoint of the effects of conformation on rates of reactions. Their work introduced the use of 4-*t*-butylcyclohexyl derivatives in which the steric bulk of the *t*-butyl group is a remote control effectively fixing the conformation of the cyclohexyl ring. The paper of Winstein and Holness showing that *cis*- and *trans*-4-*t*-butylcyclohexanol constitute a chromatographically resolvable pair of epimers having pure axial and pure equatorial C–OH, respectively, suggested the idea that an alternative method for the quantitative study of conformational equilibria might be feasible using infrared spectral techniques, inasmuch as it was well known that axial and equatorial bonds differ in their infrared stretching frequencies.[5] This involves the assumption that the corresponding C–OH stretching absorptivities in cyclohexanol (I) and the 4-*t*-butylcyclohexanols (II and III) are the same. It was hoped that the validity of this assumption could be determined by measuring the concentration of axial C–OH in cyclohexanol using the *cis* epimer concurrently with a determination of the concentration of equatorial C–OH using the *trans* epimer and checking whether or not the results were self-consistent.



I



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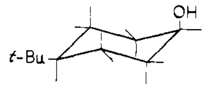

cis

III

Results and Discussion

A preliminary survey of the spectra of cyclohexanol and the pair of 4-*t*-butyl derivatives was promising, and a quantitative infrared analysis was accordingly undertaken. On dilution and more careful analysis, it readily was apparent, however, that the absorption in cyclohexanol corresponding to the strongest absorption in *cis*-4-*t*-butylcyclohexanol at 955 cm.$^{-1}$ is complex, undoubtedly due to coupling between the C–OH frequency and one of the numerous skeletal frequencies in this spectral region. A comparison of the absorption in cyclohexanol corresponding to that in the *trans* epimer alone accordingly was carried out, although the self-consistency of the system could not be demon-

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[1] Presented at the Delaware Valley Regional Meeting, American Chemical Society, Philadelphia, February 5, 1958.

[2] For general reviews: W. G. Dauben and K. S. Pitzer, in "Steric Effects in Organic Chemistry," M. Newman, ed., John Wiley and Sons, Inc., New York, N. Y., 1956, Ch. 1; D. H. R. Barton and R. C. Cookson, *Quart. Revs.*, **10**, 44 (1956); W. Klyne, "Progress in Stereochemistry," Vol. I, Buttersworth Scientific Publications, London, 1954, Ch. 2; H. D. Orloff, *Chem. Revs.*, **54**, 347 (1954).

[3] S. Winstein and N. J. Holness, THIS JOURNAL, **77**, 5562 (1955).

[4] (a) E. L. Eliel and R. S. Ro, *Chemistry & Industry*, 251 (1956); THIS JOURNAL, **79**, 5995 (1957); (b) E. L. Eliel and C. A. Lukach, *ibid.*, **79**, 5986 (1957).

[5] *Cf.* R. N. Jones and C. Sandorfy, in "Chemical Applications of Spectroscopy," W. West, ed., Vol. IX, "Technique of Organic Chemistry," A. Weissberger, ed., Interscience Publishers, Inc., New York, N. Y., 1956, Ch. 4; E. A. Braude and E. S. Waight, in "Progress in Stereochemistry," Vol. I, W. Klyne, ed., Academic Press, New York, N. Y., 1954, Ch. 4.